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SEQUENCE LISTING

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Chow, Yen-Hung
Phogat, Sanjay Kumar
Broder, Christopher Charles
The Government of the United States of America
as represented by The Secretary of the
Department of Health and Human Services

<120> HIV-1 Envelope Glycoproteins Stabilized by Flexible
Linkers as Potent Entry Inhibitors and Immunogens

<130> 015280-458000US

<140> US 10/506,651

<141> 2004-09-02

<150> WO PCT/US02/07144

<151> 2002-03-05

<160> 33

<170> PatentIn Ver. 2.1

<210> 1

<211> 2562

<212> DNA

<213> Human immunodeficiency virus type 1

<220>

<223> full length human immunodeficiency virus-1 (HIV-1)
envelope glycoprotein (Env, gp160) from primary
R5X4 isolate 89.6

<400> 1

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gatacagagg tacataatgt ttgggccaca catgcctgtg tacccacaga cccaaccca 240
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gatcagatgc atcgagatat aatcagttta tgggatgaaa gcctaaagcc atgtgtaaaa 360
ttaaccccac tctgtgttac tttaaattgc actaatttga atatcactaa gaatactact 420
aatcccacta gtagcagctg gggaatgatg gagaaaggag aaataaaaaa ttgctctttc 480
tatatcacca caagcataag aaataaggta aagaaagaat atgcactttt taatagactt 540
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gagactgaga tcttcagacc tggaggagga gatatgaggg acaattggag aagtgaatta 1440
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<210> 2

<211> 853

<212> PRT

<213> Human immunodeficiency virus type 1

<220>

<223> full length human immunodeficiency virus-1 (HIV-1)
 envelope glycoprotein (Env, gp160) from primary
 R5X4 isolate 89.6

<400> 2

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Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
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His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
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Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro
195 200 205
Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro Ala Gly
210 215 220
Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser Gly Pro
225 230 235 240
Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val
245 250 255
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260 265 270
Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val
275 280 285
Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn
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Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg
305 310 315 320
Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg
325 330 335
Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu
340 345 350
Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly Gly Asp
355 360 365
Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr
370 375 380
Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly Gly
385 390 395 400
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405 410 415
Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met Tyr Ala
420 425 430
Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu
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 His Met Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg
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 Val Leu Ala Leu Glu Arg Tyr Leu Arg Asp Gln Gln Leu Met Gly Ile
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 Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ser Val Pro Trp Asn
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 610 615 620
 Trp Met Glu Trp Glu Arg Glu Ile Asp Asn Tyr Thr Asp Tyr Ile Tyr
 625 630 635 640
 Asp Leu Leu Glu Lys Ser Gln Thr Gln Gln Glu Lys Asn Glu Lys Glu
 645 650 655
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 Thr Asn Trp Leu Trp Tyr Ile Arg Leu Phe Ile Met Ile Val Gly Gly
 675 680 685
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 690 695 700
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 740 745 750
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 770 775 780
 Arg Gly Trp Glu Ala Leu Lys Tyr Trp Trp Asn Leu Leu Gln Tyr Trp
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Ser Gln Glu Leu Lys Asn Ser Ala Val Ser Leu Leu Asn Ala Thr Ala
805 810 815

Ile Ala Val Ala Glu Gly Thr Asp Arg Val Ile Lys Ile Val Gln Arg
820 825 830

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Glu Arg Ala Leu Leu
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<210> 3
<211> 2051
<212> DNA
<213> Human immunodeficiency virus type 1

<220>
<223> human immunodeficiency virus-1 (HIV-1) envelope
glycoprotein gp140 truncated version of gp 160
from primary R5X4 isolate 89.6

<400> 3

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| gtacctgtgt | ggagagaagc | aaccaccact | ctattttgtg | catcagatgc | taaagcctat | 180 |
| gatacagagg | tacataatgt | ttgggccaca | catgcctgtg | taccacaga | ccccaacca | 240 |
| caagaagtag | tattgggaaa | tgtgacagaa | aattttaaca | tgtggaaaa | taacatggta | 300 |
| gatcagatgc | atgaggatat | aatcagttta | tgggatgaaa | gcctaaagcc | atgtgtaaaa | 360 |
| ttaaccccac | tctgtgttac | tttaaattgc | actaatttga | atatcactaa | gaatactact | 420 |
| aatcccacta | gtagcagctg | gggaatgatg | gagaaaggag | aaataaaaaa | ttgctctttc | 480 |
| tatatcacca | caagcataag | aaataaggta | aagaaagaat | atgcactttt | taatagactt | 540 |
| gatgtagtac | caatagaaaa | tactaataat | actaagtata | ggttaataag | ttgtaacacc | 600 |
| tcagtcatta | cacaggcctg | tccaaaggta | tcctttcagc | caattcccat | acattattgt | 660 |
| gtcccggtcg | ggtttgcgat | gctaaagtgt | aacaataaga | cattcaatgg | atcaggacca | 720 |
| tgcacaaatg | tcagcacagt | acaatgtaca | catggaatta | ggccagtggg | gtcaactcaa | 780 |
| ctgctgttaa | atggcagctc | agcagaagaa | gacatagtaa | ttagatctga | aaatttcaca | 840 |
| gacaatgcta | aaaccataat | agtacagcta | aatgaatctg | tagtaattaa | ttgtacaaga | 900 |
| cccaacaaca | atacaagaag | aaggttatct | ataggaccag | ggagagcatt | ttatgcaaga | 960 |
| agaaacataa | taggagatat | aagacaagca | cattgtaaca | ttagtagagc | aaaatggaat | 1020 |
| aacacttttac | aacagatagt | tataaaatta | agagaaaaat | ttaggaataa | aacaatagcc | 1080 |
| tttaatcaat | cctcaggagg | ggaccagaaa | attgtaatgc | acagttttta | ttgtggaggg | 1140 |
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| acaaatggca | ctgaaggaaa | tgacataatc | acactccaat | gcagaataaa | acaaattata | 1260 |
| aatatgtggc | agaaagtagg | aaaagcaatg | tatgcccctc | ccatcacagg | acaaattaga | 1320 |
| tgttcatcaa | atattacagg | gctgctacta | acaagagatg | gaggtaatag | tactgagact | 1380 |
| gagactgaga | tcttcagacc | tggaggagga | gatatgaggg | acaattggag | aagtgaatta | 1440 |
| tataaatata | aagtagtaag | aattgaacca | ataggagtag | caccaccag | ggcaaagaga | 1500 |
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| ttattgtctg | gtatagtga | gcagcagaaa | aatctgctga | gggctattga | ggcgcaacag | 1680 |
| catatgttgc | aactcacagt | ctggggcatc | aagcagctcc | aggcaagagt | cctggctctg | 1740 |
| gaaagatacc | taagggatca | acagctcatg | ggaatttggg | gttgctctgg | aaaactcatt | 1800 |
| tgcaccactt | ctgtgccttg | gaatgttagt | tggagtaata | aatctgtgga | tgatatttgg | 1860 |
| aataacatga | cctggatgga | gtgggaaaga | gaaattgaca | attacacaga | ctatatatat | 1920 |
| gacttacttg | aaaaatcgca | aacccaacaa | gaaaagaatg | aaaaagaatt | attggaattg | 1980 |
| gataaatggg | caagtttgtg | gaattgggtt | gacataacaa | actggctgtg | gtatataaga | 2040 |
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<210> 4
 <211> 667
 <212> PRT
 <213> Human immunodeficiency virus type 1

 <220>
 <223> human immunodeficiency virus-1 (HIV-1) envelope
 glycoprotein gp140 truncated version of gp 160
 from primary R5X4 isolate 89.6

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 35 40 45
 Thr Thr Leu Phe Cys Ala Ser Asp Ala Lys Ala Tyr Asp Thr Glu Val
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 His Asn Val Trp Ala Thr His Ala Cys Val Pro Thr Asp Pro Asn Pro
 65 70 75 80
 Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn Met Trp Lys
 85 90 95
 Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser Leu Trp Asp
 100 105 110
 Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys Val Thr Leu
 115 120 125
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 180 185 190
 Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln Ala Cys Pro
 195 200 205
 Lys Val Ser Phe Gln Pro Ile Pro Ile His Tyr Cys Val Pro Ala Gly
 210 215 220
 Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly Ser Gly Pro
 225 230 235 240
 Cys Thr Asn Val Ser Thr Val Gln Cys Thr His Gly Ile Arg Pro Val
 245 250 255
 Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu Glu Asp Ile
 260 265 270

Val Ile Arg Ser Glu Asn Phe Thr Asp Asn Ala Lys Thr Ile Ile Val
275 280 285
Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro Asn Asn Asn
290 295 300
Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe Tyr Ala Arg
305 310 315 320
Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn Ile Ser Arg
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Ala Lys Trp Asn Asn Thr Leu Gln Gln Ile Val Ile Lys Leu Arg Glu
340 345 350
Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser Gly Gly Asp
355 360 365
Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu Phe Phe Tyr
370 375 380
Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Trp Asn Val Thr Gly Gly
385 390 395 400
Thr Asn Gly Thr Glu Gly Asn Asp Ile Ile Thr Leu Gln Cys Arg Ile
405 410 415
Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala Met Tyr Ala
420 425 430
Pro Pro Ile Thr Gly Gln Ile Arg Cys Ser Ser Asn Ile Thr Gly Leu
435 440 445
Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu Thr Glu Ile
450 455 460
Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg Ser Glu Leu
465 470 475 480
Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val Ala Pro Thr
485 490 495
Arg Ala Lys Arg Arg Thr Val Gln Arg Glu Lys Arg Ala Val Gly Ile
500 505 510
Gly Ala Val Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly
515 520 525
Ala Ala Ser Val Thr Leu Thr Val Gln Ala Arg Leu Leu Leu Ser Gly
530 535 540
Ile Val Gln Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln
545 550 555 560
His Met Leu Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg
565 570 575
Val Leu Ala Leu Glu Arg Tyr Leu Arg Asp Gln Gln Leu Met Gly Ile
580 585 590

Trp Gly Cys Ser Gly Lys Leu Ile Cys Thr Thr Ser Val Pro Trp Asn
 595 600 605
 Val Ser Trp Ser Asn Lys Ser Val Asp Asp Ile Trp Asn Asn Met Thr
 610 615 620
 Trp Met Glu Trp Glu Arg Glu Ile Asp Asn Tyr Thr Asp Tyr Ile Tyr
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<210> 5
 <211> 27
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:cleavage site
 and start of gp41 in gp160 (env 89.6)

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27

<210> 6
 <211> 8
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:cleavage site
 and start of gp41 in gp160 (env 89.6)

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<210> 7
 <211> 667
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:gp140-26
 (soluble, secreted protein)

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 Thr Glu Val His Asn Gly Trp Ala Thr His Ala Cys Val Ala Thr Asp
 35 40 45

Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn
 50 55 60
 Met Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser
 65 70 75 80
 Leu Leu Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys
 85 90 95
 Val Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn
 100 105 110
 Pro Thr Ser Ser Ser Leu Gly Met Met Glu Lys Gly Glu Ile Lys Asn
 115 120 125
 Cys Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu
 130 135 140
 Tyr Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn
 145 150 155 160
 Asn Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln
 165 170 175
 Ala Cys Pro Lys Val Phe Phe Gln Pro Ile Ala Ile His Tyr Cys Val
 180 185 190
 Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly
 195 200 205
 Ser Gly Pro Cys Thr Asn Val Ser Thr Val Pro Cys Thr His Gly Ile
 210 215 220
 Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu
 225 230 235 240
 Glu Asp Ile Val Ile Arg Ser Gly Asn Phe Thr Asp Asn Ala Lys Thr
 245 250 255
 Ile Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro
 260 265 270
 Asn Asn Asn Thr Arg Arg Arg Leu Ser Ile Gly Pro Gly Arg Ala Phe
 275 280 285
 Tyr Ala Arg Arg Asn Ile Ile Gly Asp Ile Arg Gln Ala His Cys Asn
 290 295 300
 Ile Ser Arg Ala Lys Leu Asn Asn Thr Leu Gln Gln Ile Val Ile Lys
 305 310 315 320
 Leu Arg Glu Lys Phe Arg Asn Lys Thr Ile Ala Phe Asn Gln Ser Ser
 325 330 335
 Gly Gly Asp Pro Glu Ile Val Met His Ser Phe Asn Cys Gly Gly Glu
 340 345 350
 Phe Phe Tyr Cys Asn Thr Ala Gln Leu Phe Asn Ser Thr Leu Asn Val
 355 360 365

Thr Gly Gly Thr Asn Gly Thr Glu Glu Asn Asp Ile Ile Thr Leu Gln
 370 375 380
 Cys Arg Ile Lys Gln Ile Ile Asn Met Trp Gln Lys Val Gly Lys Ala
 385 390 395 400
 Met Tyr Ala Pro Pro Ile Thr Gly Gln Ile Ile Cys Ser Ser Asn Ile
 405 410 415
 Thr Gly Leu Leu Leu Thr Arg Asp Gly Gly Asn Ser Thr Glu Thr Glu
 420 425 430
 Thr Glu Ile Phe Arg Pro Gly Gly Gly Asp Met Arg Asp Asn Trp Arg
 435 440 445
 Ser Glu Leu Tyr Lys Tyr Lys Val Val Arg Ile Glu Pro Ile Gly Val
 450 455 460
 Ala Pro Thr Arg Ala Lys Arg Arg Thr Cys Gln Gly Gly Ile Asp Gly
 465 470 475 480
 Ile Leu Gln Ile Ser Gly Ser Gly Ser Gly Gly Ser Gly Gln Gly Ser
 485 490 495
 Ser Ser Gly Gly Ala Gly Gly Lys Gly Ala Val Gly Ile Gly Ala Val
 500 505 510
 Phe Leu Gly Phe Leu Gly Ala Ala Gly Ser Thr Met Gly Ala Arg Ser
 515 520 525
 Val Thr Leu Thr Val Gln Ala Arg Leu Leu Leu Ser Gly Ile Val Gln
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 Gln Gln Asn Asn Leu Leu Arg Ala Ile Glu Ala Gln Gln His Met Leu
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 Gln Leu Thr Val Trp Gly Ile Lys Gln Leu Gln Ala Arg Val Leu Ala
 565 570 575
 Leu Glu Arg Tyr Leu Arg Asp Gln Gln Leu Met Gly Ile Trp Gly Cys
 580 585 590
 Ser Gly Lys Leu Ile Cys Thr Thr Ser Val Pro Trp Asn Val Ser Trp
 595 600 605
 Ser Asn Lys Ser Val Asp Asp Ile Trp Asn Asn Met Thr Trp Met Glu
 610 615 620
 Leu Glu Arg Glu Ile Asp Asn Tyr Thr Asp Tyr Ile Tyr Asp Leu Leu
 625 630 635 640
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 645 650 655
 Leu Asp Lys Trp Ala Ser Leu Trp Lys Leu Val
 660 665

<210> 8
 <211> 656
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:gp140-15
 (soluble, secreted protein)

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 35 40 45
 Pro Asn Pro Gln Glu Val Val Leu Gly Asn Val Thr Glu Asn Phe Asn
 50 55 60
 Met Trp Lys Asn Asn Met Val Asp Gln Met His Glu Asp Ile Ile Ser
 65 70 75 80
 Leu Leu Asp Glu Ser Leu Lys Pro Cys Val Lys Leu Thr Pro Leu Cys
 85 90 95
 Val Thr Leu Asn Cys Thr Asn Leu Asn Ile Thr Lys Asn Thr Thr Asn
 100 105 110
 Pro Thr Ser Ser Ser Leu Gly Met Met Glu Lys Gly Glu Ile Lys Asn
 115 120 125
 Cys Ser Phe Tyr Ile Thr Thr Ser Ile Arg Asn Lys Val Lys Lys Glu
 130 135 140
 Tyr Ala Leu Phe Asn Arg Leu Asp Val Val Pro Ile Glu Asn Thr Asn
 145 150 155 160
 Asn Thr Lys Tyr Arg Leu Ile Ser Cys Asn Thr Ser Val Ile Thr Gln
 165 170 175
 Ala Cys Pro Lys Val Phe Phe Gln Pro Ile Ala Ile His Tyr Cys Val
 180 185 190
 Pro Ala Gly Phe Ala Met Leu Lys Cys Asn Asn Lys Thr Phe Asn Gly
 195 200 205
 Ser Gly Pro Cys Thr Asn Val Ser Thr Val Pro Cys Thr His Gly Ile
 210 215 220
 Arg Pro Val Val Ser Thr Gln Leu Leu Leu Asn Gly Ser Leu Ala Glu
 225 230 235 240
 Glu Asp Ile Val Ile Arg Ser Gly Asn Phe Thr Asp Asn Ala Lys Thr
 245 250 255
 Ile Ile Val Gln Leu Asn Glu Ser Val Val Ile Asn Cys Thr Arg Pro
 260 265 270

| | | | | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Asn | Asn | Asn | Thr | Arg | Arg | Arg | Leu | Ser | Ile | Gly | Pro | Gly | Arg | Ala | Phe | 275 | 280 | 285 |
| Tyr | Ala | Arg | Arg | Asn | Ile | Ile | Gly | Asp | Ile | Arg | Gln | Ala | His | Cys | Asn | 290 | 295 | 300 |
| Ile | Ser | Arg | Ala | Lys | Leu | Asn | Asn | Thr | Leu | Gln | Gln | Ile | Val | Ile | Lys | 305 | 310 | 315 |
| Leu | Arg | Glu | Lys | Phe | Arg | Asn | Lys | Thr | Ile | Ala | Phe | Asn | Gln | Ser | Ser | 325 | 330 | 335 |
| Gly | Gly | Asp | Pro | Glu | Ile | Val | Met | His | Ser | Phe | Asn | Cys | Gly | Gly | Glu | 340 | 345 | 350 |
| Phe | Phe | Tyr | Cys | Asn | Thr | Ala | Gln | Leu | Phe | Asn | Ser | Thr | Leu | Asn | Val | 355 | 360 | 365 |
| Thr | Gly | Gly | Thr | Asn | Gly | Thr | Glu | Glu | Asn | Asp | Ile | Ile | Thr | Leu | Gln | 370 | 375 | 380 |
| Cys | Arg | Ile | Lys | Gln | Ile | Ile | Asn | Met | Trp | Gln | Lys | Val | Gly | Lys | Ala | 385 | 390 | 395 |
| Met | Tyr | Ala | Pro | Pro | Ile | Thr | Gly | Gln | Ile | Ile | Cys | Ser | Ser | Asn | Ile | 405 | 410 | 415 |
| Thr | Gly | Leu | Leu | Leu | Thr | Arg | Asp | Gly | Gly | Asn | Ser | Thr | Glu | Thr | Glu | 420 | 425 | 430 |
| Thr | Glu | Ile | Phe | Arg | Pro | Gly | Gly | Gly | Asp | Met | Arg | Asp | Asn | Trp | Arg | 435 | 440 | 445 |
| Ser | Glu | Leu | Tyr | Lys | Tyr | Lys | Val | Val | Arg | Ile | Glu | Pro | Ile | Gly | Val | 450 | 455 | 460 |
| Ala | Pro | Thr | Arg | Ala | Lys | Arg | Arg | Thr | Cys | Gln | Gly | Gly | Ile | Asp | Gly | 465 | 470 | 475 |
| Ile | Leu | Gln | Ile | Ser | Ser | Ser | Gly | Gly | Ala | Gly | Gly | Lys | Gly | Ala | Val | 485 | 490 | 495 |
| Gly | Ile | Gly | Ala | Val | Phe | Leu | Gly | Phe | Leu | Gly | Ala | Ala | Gly | Ser | Thr | 500 | 505 | 510 |
| Met | Gly | Ala | Arg | Ser | Val | Thr | Leu | Thr | Val | Gln | Ala | Arg | Leu | Leu | Leu | 515 | 520 | 525 |
| Ser | Gly | Ile | Val | Gln | Gln | Gln | Asn | Asn | Leu | Leu | Arg | Ala | Ile | Glu | Ala | 530 | 535 | 540 |
| Gln | Gln | His | Met | Leu | Gln | Leu | Thr | Val | Trp | Gly | Ile | Lys | Gln | Leu | Gln | 545 | 550 | 555 |
| Ala | Arg | Val | Leu | Ala | Leu | Glu | Arg | Tyr | Leu | Arg | Asp | Gln | Gln | Leu | Met | 565 | 570 | 575 |
| Gly | Ile | Trp | Gly | Cys | Ser | Gly | Lys | Leu | Ile | Cys | Thr | Thr | Ser | Val | Pro | 580 | 585 | 590 |

Trp Asn Val Ser Trp Ser Asn Lys Ser Val Asp Asp Ile Trp Asn Asn
595 600 605

Met Thr Trp Met Glu Leu Glu Arg Glu Ile Asp Asn Tyr Thr Asp Tyr
610 615 620

Ile Tyr Asp Leu Leu Glu Lys Ser Gln Thr Gln Gln Glu Lys Asn Glu
625 630 635 640

Lys Glu Leu Leu Glu Leu Asp Lys Trp Ala Ser Leu Trp Lys Leu Val
645 650 655

<210> 9
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:peptide linker

<400> 9
Gly Ile Leu Ile
1

<210> 10
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:preferred
peptide linker

<400> 10
Gly Gly Ile Asp Gly Ile Leu Gln Ile Ser Ser Ser Gly Gly Ala
1 5 10 15

<210> 11
<211> 26
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:preferred
peptide linker

<400> 11
Gly Gly Ile Asp Gly Ile Leu Gln Ile Ser Gly Ser Gly Ser Gly Gly
1 5 10 15

Ser Gly Gln Gly Ser Ser Ser Gly Gly Ala
20 25

<210> 12
<211> 15
<212> PRT
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:preferred
peptide linker

<400> 12

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
1 5 10 15

<210> 13

<211> 20

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:preferred
peptide linker

<400> 13

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly
20

<210> 14

<211> 25

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:preferred
peptide linker

<400> 14

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Ser Gly Gly
20 25

<210> 15

<211> 24

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<220>

<221> MOD_RES

<222> (7)..(24)

<223> amino acids at positions 7-8, 9-10, 11-12, 13-14,
15-16, 17-18, 19-20, 21-22, and 23-24 may be
present or absent

<400> 15
 Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
 1 5 10 15

Gly Ser Gly Ser Gly Ser Gly Ser
 20

<210> 16
 <211> 48
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:exemplary
 linker

<220>
 <221> MOD_RES
 <222> (13)..(48)
 <223> amino acids at positions 13-16, 17-21, 22-24,
 25-28, 29-32, 33-36, 37-40, 41-44, and 45-48 may
 be present or absent

<400> 16
 Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 1 5 10 15
 Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 20 25 30
 Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 35 40 45

<210> 17
 <211> 60
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:exemplary
 linker

<220>
 <221> MOD_RES
 <222> (16)..(60)
 <223> amino acids at positions 16-20, 21-25, 26-30,
 31-35, 36-40, 41-45, 46-50, 51-55 and 55-60 may be
 present or absent

<400> 17
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
 /1 5 10 15
 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 20 25 30

Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly
 35 40 45

Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 50 55 60

<210> 18
 <211> 72
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:exemplary
 linker

<220>
 <221> MOD_RES
 <222> (19)..(72)
 <223> amino acids at positions 19-24, 25-30, 31-36,
 37-42, 43-48, 49-54, 55-60, 61-66 and 67-72 may be
 present or absent

<400> 18
 Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
 1 5 10 15
 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly
 20 25 30
 Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
 35 40 45
 Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
 50 55 60
 Gly Ser Gly Gly Gly Gly Ser
 65 70

<210> 19
 <211> 12
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:exemplary
 linker

<400> 19
 Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 1 5 10

<210> 20
 <211> 20
 <212> PRT
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 20

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly
1 5 10 15

Gly Gly Gly Ser
20

<210> 21

<211> 30

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 21

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Ser
20 25 30

<210> 22

<211> 42

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 22

Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Ser Gly Gly
1 5 10 15

Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
20 25 30

Gly Gly Ser Gly Gly Gly Gly Gly Ser
35 40

<210> 23

<211> 56

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 23

Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Ser
1 5 10 15

Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Ser
20 25 30

Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Ser
35 40 45

Gly Gly Gly Gly Gly Gly Gly Ser
50 55

<210> 24

<211> 72

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 24

Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly
1 5 10 15

Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly
20 25 30

Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly
35 40 45

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly
50 55 60

Gly Gly Gly Gly Gly Gly Gly Ser
65 70

<210> 25

<211> 90

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 25

Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly
1 5 10 15

Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly
20 25 30

Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly
35 40 45

Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
50 55 60

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser
65 70 75 80

Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser
85 90

<210> 26

<211> 110

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 26

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly
1 5 10 15

Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
20 25 30

Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
35 40 45

Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
50 55 60

Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly
65 70 75 80

Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly
85 90 95

Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser
100 105 110

<210> 27

<211> 132

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence:exemplary
linker

<400> 27

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
1 5 10 15

Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly
20 25 30

Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser
35 40 45

Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
50 55 60

Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly
 65 70 75 80
 Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser
 85 90 95
 Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly
 100 105 110
 Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly
 115 120 125
 Gly Gly Gly Ser
 130

<210> 28
 <211> 156
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Description of Artificial Sequence:exemplary
 linker

<400> 28
 Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly
 1 5 10 15
 Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly
 20 25 30
 Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
 35 40 45
 Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
 50 55 60
 Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly
 65 70 75 80
 Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly
 85 90 95
 Gly Gly Gly Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly
 100 105 110
 Gly Gly Gly Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly
 115 120 125
 Gly Ser Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser Gly
 130 135 140
 Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Gly Ser
 145 150 155

<210> 29
 <211> 36
 <212> PRT
 <213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:exemplary
linker

<220>
<221> MOD_RES
<222> (10)..(36)
<223> amino acids at positions 10-12, 13-15, 16-18,
19-21, 22-24, 25-27, 28-30, 31-33 and 34-36 may be
present or absent

<400> 29
Ala Gly Ser Ala Gly Ser Ala Gly Ser Ala Gly Ser Ala
1 5 10 15
Gly Ser Ala Gly Ser Ala Gly Ser Ala Gly Ser Ala Gly
20 25 30
Ser Ala Gly Ser
35

<210> 30
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:post
translational cleavage site

<400> 30
Arg Glu Lys Arg
1

<210> 31
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:mutated post
translational cleavage site

<400> 31
Arg Glu Ile Asp
1

<210> 32
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:short fragment
following mutated cleavage site created by
introduction of restriction sites

<400> 32
Glu Phe Ile Ser
1

<210> 33
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: polypeptide
linker end

<400> 33
Gly Gly Ser Gly Gly
1 5